DOI:
10.1039/C8FO00729B

Document Version
Peer reviewed version

Food & Function

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: R. Jawad, G. P. Martin, C. Elleman and P. G. Royall, *Food Funct.*, 2018, DOI: 10.1039/C8FO00729B.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

View Article Online
DOI: 10.1039/C8FO00729B

Crystallisation of freeze-dried sucrose in model mixtures that represent the amorphous sugar matrices present in confectionery.

Rim Jawad[1], Carole Elleman[2], Gary P. Martin[1] and Paul G. Royall[1,*]

Addresses:

[1]Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK.

[2]The Reading Science Centre, Reading Scientific Services Ltd., Whiteknights Campus, Pepper Lane, Reading RG6 6LA, UK.

Keywords:

Sucrose, lactose, amorphous, crystallisation, freeze-drying, polarimetry, glass transition temperature

Abstract

Recrystallisation occurs frequenctly in confectionery. More information on sucrose re-crystallisation will aid our understanding of popular foods like chocolate. However, progress has been limited due the lack of a robust method for the production of amorphous sucrose, with known purity. Poor control has led to the glass transition temperatures (T_g's) for amorphous sucrose varying between 48-78°C in the literature. Our objective was to investigate the recrystallization of sucrose in the presence of lactose, NaCl and water. The purity of sucrose was confirmed by ion chromatography, polarimetry and differential scanning calorimetry. Amorphous sucrose was prepared by freeze-drying 10% w/v aqueous solutions. Fisher (99.7%) and SilverSpoon (98.4%) sucrose samples melted at 186±0.6°C & 189±0.3°C respectively. For the Fisher sample the absence of invert sugars and low mineral content allowed the observation of a small endotherm (≈150°C). The T_g of amorphous sucrose was 58.3±1.1°C with a recrystallization enthalpy (ΔH_{crys}) of 72.8±6.0J/g. NaCl reduced both the T_g (54.8±1.8°C) and the ΔH_{crys} (35.7±3.8J/g) without affecting the onset temperature of sucrose's re-crystallization (T_{crys},

View Article Online
DOI: 10.1039/C8FO00729B

129.5±6.9°C), suggesting that a proportion of the sample remained amorphous. The presence of water (1.6±0.07%) inside the hermetically sealed pans caused an earlier onset of T_g (52.3±1.3°C) and T_{crys} (85.1±4.0°C), as well as lowering ΔH_{crys} (45.2±2.4J/g) compared to samples contained in pin-holed pans (where evaporation was possible). The presence of lactose inhibited the crystallization of sucrose completely. On the basis of this study, it is apparent that sucrose crystallization is highly dependent on the presence of other common food ingredients within the matrix.

1. Introduction

The major ingredient of milk chocolate by weight is sucrose (Table 1). Sucrose and lactose are introduced at the mixing stage of milk chocolate production after the cocoa butter and cocoa liquor have been extracted from the roasted cocoa beans (Figure 1). Subsequent refining of this mixture, to reduce sucrose particle and aggregate size, typically involves roller mills which apply high mechanical stress on the forming chocolate mass. Comminution, especially milling, introduces disorder on the surface of both sucrose and lactose particles[1-3]. Further occurrence of amorphous solids within confectionery is likely because spray-dried milk powder is often used as the vehicle for introducing lactose into the mixture. Lactose present in spray-dried milk powder is in a relatively stable amorphous form[4,5].

The recrystallisation of sugars from a melt is a commonly used process throughout many confectionery manufacturing processes, but the influence of lactose and other common ingredients on such recrystallisation has not been investigated systematically in previous studies. To produce the required amorphous mixtures for the current work, it was essential to develop a robust method for the production of amorphous sucrose (with known purity).

Spray-drying has been widely used in both food and pharmaceutical manufacturing processes, where the rapid removal of the solvent inhibits crystallisation of the solute and results in the formation of an amorphous solid. For example, spray-drying of aqueous solutions of sucrose using an inlet temperature of 165 °C and outlet 104 °C at a feed concentration of 17%

View Article Online
DOI: 10.1039/C8FO00729B

w/v generates a sucrose glass.[6] Such processes are difficult to control as a consequence of the glass transition temperature of sucrose being relatively low (approximately 50°C) and as a result of the thermal degradation of sucrose, which is reported to occur above 150 °C[6]. Therefore, the formation of amorphous sucrose by spray-drying is limited by its low glass transition[7,8] and little consideration has been given to the thermal degradation or hydrolysis of sucrose upon exposure to high temperatures in many of the reported spray-drying studies carried out to date. A lack of appreciation of potential impurities, and poorly controlled water content, has probably led to the large variance in the reported values for the glass transition temperature of sucrose, between 48 and 78 °C (Table 2).

Freeze-drying is a more benign process compared to spray-drying, as it avoids excessive heat but still has the capability of producing amorphous sugars[8-12]. The sugar glasses produced by freeze-drying show slightly different crystallization kinetics from amorphous sucrose samples prepared by spray drying and milling[3,8]. Spray drying and milling are used in confectionery manufacture, however as their application has the potential for generating degradation products, the method of freeze-drying was applied in the study reported here. Although not an exact match for the crystallization of amorphous sugars in confectionery, freeze dried systems are potentially a simple way of modeling the recrystallization of amorphous sucrose in the presence of other ingredients. Even though freeze-drying is considered to have low potential for thermal degradation it is a long process; typically taking 2-5 days to effect. Therefore, special attention was paid in the work reported here to check for inversion to ensure that the freeze-dried product contains pure sucrose absent of invert sugars. Sodium chloride was included in the model sugar mixtures to investigate the influence of a typical salt on crystallisation. Accordingly, the aim of this work was to investigate systematically the crystallisation of sucrose in the presence of lactose, NaCl and residual water.

View Article Online
DOI: 10.1039/C8FO00729B

2.1 Materials

SigmaUltra sucrose ≥ 99.5%, α-lactose monohydrate, sodium chloride, D-(+)-glucose ≥99.5%, D-(-)-fructose ≥99% and 6 M hydrochloric acid solution were purchased from Sigma-Aldrich (Sigma-Aldrich Company Ltd., England). *Silver Spoon* sugar and sucrose (AR Grade) were purchased from the Silver Spoon Company and Fisher Scientific (United Kingdom) respectively.

2.2 Methods

2.2.1 Preparation of the feed solutions for freeze-drying

For freezing drying, 10% w/v aqueous feed solutions of either sucrose, sucrose with α-lactose monohydrate or sucrose with NaCl (present in different concentrations) were prepared in HPLC grade water. The 10% w/v feed solution is with respect to all the solid material added. All solutions were freshly prepared and stored at 25 °C. Five groups of 10% w/v aqueous solutions were prepared to give the following concentrations:

- 100% w/w sucrose with respect to the final freeze-dried sample; low level of NaCl & low level of lactose (LL)
- 66% w/w sucrose and 34% w/w lactose; low level of NaCl & high level of lactose (LH)
- 97% w/w sucrose and 3% w/w NaCl; high level of NaCl & low level of lactose (HL)
- 81.5% w/w sucrose, 17% w/w lactose and 1.5% w/w NaCl; medium level of NaCl & medium level of lactose (MM)
- 63% w/w sucrose, 34% w/w lactose and 3% w/w NaCl; high level of NaCl & high level of lactose (HH)

2.2.2 Freeze-drying

Sucrose solutions were freeze-dried in vials using Varian Girovac model GVD4 freeze-dryer. A 10% w/v sucrose aqueous solution was frozen at -80 °C for 2 h then subjected to primary drying at -50 °C for 72 h followed by secondary drying over phosphorous pentoxide,

View Article Online
DOI: 10.1039/C8FO00729B

P_2O_5 for 48 h, where no further water loss was detected as evidenced by constant mass. Immediately after secondary drying, the vials were sealed under a nitrogen atmosphere and the sealed vials were stored in a desiccator over P_2O_5 at controlled room temperature (25 °C). The intention of this method was to ensure that no change in the water content within the stored materials occurred during and up to 24 weeks of storage. The samples were tested, for example by differential scanning calorimetry, at t_0 and after 1, 4, 12 and 24 weeks with aim of investigating the impact of storage time on the thermal properties of the freeze-dried samples. A large batch of sample vials was produced and placed into storage. Once a particular vial was removed from storage and opened, it was not returned, the batch size allowed an unopened vial to be sacrificed for each time point.

2.2.3 Water content determination

The Karl-Fisher (Metrohm 870 KF Titrino) was standardised using the Karl-Fisher reagent hydranal standard (5-Standard sodium tartrate dihydrate)[13], the measurement being made in triplicate. Samples (0.2 g) were then analysed in duplicate using hydranal Composite 5, and a solvent mixture of 1:1:1 methanol: formamide: chloroform.

2.2.4 Thermogravimetric analysis (TGA)

A PerkinElmer Pyris 6 thermogravimetric analysis (TGA) instrument was calibrated for temperature and weight according to the TGA manual supplied by the manufacturer. Samples of 5 - 10 mg of sucrose were loaded into an open pan using a micro-spatula and heated at a heating rate of 10 ºC/min over a temperature range of 25-150 ºC, with the sample mass measured as a function of temperature and time.

2.2.5 Determination of the purity of crystalline sucrose by ion chromatography (IC)

A Dionex ion chromatography system (ICS) 3000 was used, comprising a Carbopack column attached to a Dionex Chromeleon (version 6.80) as recorder. The mobile phase (flow rate of 1 mL/min) was milli-Q water and 300 mM NaOH and sucrose-containing sample

View Article Online
DOI: 10.1039/C8FO00729B

solutions were prepared by weighing approximately 0.1 g material and diluting to volume in 100 mL with milli-Q water. The mixed standards were prepared in the same manner as the sucrose solutions each containing 0.1 g of sucrose, fructose and glucose. Solutions were further diluted 10-fold and 10 μL of each solution was injected for analysis.

2.2.6 Optical rotation

The PerkinElmer polarimeter 430 was first zeroed for air and water and then calibrated for sucrose by measuring the optical rotation (OR) of a 10 g/mL of aqueous SigmaUltra sucrose solution, calculating its specific rotation and comparing it to reference values reported in the literature. Once the correct functioning of the polarimeter was confirmed, it was zeroed using a blank cell, filled with HPLC water. The samples were then measured and were comprised of the following proportions: 100% w/w sucrose, sucrose: invert sugar (75:25, 50:50, 25:75), 100% w/w invert sugar and hydrolysed sucrose (sucrose in 6-M HCL). The test procedure involved the preparation of a concentration of 10% w/v of different sucrose/invert sugar compositions. Thereafter, three replicates of freeze-dried sucrose samples were measured to detect the presence of any invert sugar in freeze-dried sucrose.

The specific rotation $[\alpha_{Obs}]_D$ was normalised for solution concentration and path-length in this study and was calculated by applying equation 2 to the measured or observed optical rotation data.

$$[\alpha_{Obs}]_D = \frac{100.\alpha}{l.C} \qquad \text{Equation 2}$$

Where $[\alpha_{Obs}]_D$ = observed specific rotation, D = sodium D line monochromatic radiation (λ = 589 nm), α = observed optical rotation, l = path-length in dm, C = concentration in g/100 mL[14].

View Article Online
DOI: 10.1039/C8FO00729B

2.2.7 Differential scanning calorimetry (DSC)

The TA Q20 DSC was calibrated according to an in-house standard operating procedure (SOP). The melting point and enthalpy of indium are 156.6 ºC and 28.71 J/g respectively were used, based on literature values. Additional DSC runs were made using dry N_2 purging and the instrument linked to a Haake EK90/MT intracooler. Mettler Toledo hermetic aluminium DSC pans (40 uL) with lids were employed.

The sample was prepared by weighing 3-5 mg of the freeze-dried sucrose-containing sample into a DSC pan, a pin-holed lid (to allow evaporation of any residual moisture embedded in the sample) was crimped into position to ensure that the pan was sealed. The DSC experiments were carried out by equilibrating the system at 25 ºC for 10 minutes, followed by a ramping rate of 10 °C/min from 25 ºC to 200 ºC. The resulting graph was analysed by the universal analysis software 2000 (TA Instruments).

Glass transition temperatures were determined using TA Instruments Universal Analysis Software; essentially this consisted of extrapolating the two baselines before and after the T_g and determining the temperature where the mid point in the step change in the heat flow signal was achieved. For the other thermal transitions, the integration of the peaks in the heat flow signal allowed the determination of the enthalpy for these transitions, Integration was obtained by linear extrapolation of the pre- and post-transition baselines over the selected temperature range.

2.2.8 Nuclear magnetic resonance (NMR)

NMR was used to confirm that the anomeric composition of lactose had reached its equilibrium value for the lactose-containing samples. NMR samples were prepared by dissolving 3-4 mg of lactose in 0.7 mL of dimethylsulfoxide (DMSO) (- d6 99.9% at %D with 0.05 % v/v tetramethylsilane (TMS) (Goss Scientific instruments Ltd)). 1-dimensional 1H spectra were recorded on a Bruker Avance 400-MHz spectrometer following the method described by Jawad *et al.*[15]. The TMS reference was used to enable comparison of any chemical shifts.

View Article Online
DOI: 10.1039/C8FO00729B

2.2.9 Hot stage microscopy (HSM)

Hot stage microscopy was carried out using a Leitz Dialux 22EB microscope fitted with crossed polarisers and a λ plate (red 1 compensator)[16], with a Qi Imaging QiFastcam attached to a computer running the Linksys32 software package (Linkam). The freeze-dried sugar was placed onto a coverslip and a second coverslip was employed to sandwich the sample. The sample was then placed into a Linkam HFS91 heated stage, which was attached to the microscope, controlled by a Linkam TP92 controller. The TP92 control box was attached to the computer, so that the camera and heated stage could both be controlled simultaneously via the Linksys software package. The sample was then heated from 25-200 °C at 2 °C/min with a picture being taken by the camera every 10 s.

2.2.10 Design of experiments (DoE)

Because there are a number of factors which influence crystallisation, an experimental strategy was warranted to tackle the concept systematically based on the approach termed design of experiment (DoE)[17,18].

The DoE was set using the software (JMP® Statistical Discovery Software, version 10, supplied by SAS Institute Inc., USA). A JMP® data table is organized as a spread sheet where columns correspond to variables and rows correspond to observations. The variables in the study reported here were lactose, NaCl and residual water. The data can be rearranged in the JMP® data table with tables, rows, and columns menus.

The randomisation of the week, day, and order is vital to eliminate any potential bias. Randomisation helps in distinguishing a 'true' experiment from a 'quasi' experiment which in turn enhances the precision of experiments.

3 Results and Discussion

The onset of melting for *'Fisher'* sucrose was found to be 186 ± 0.6 °C (± SD, n = 3) with a melting enthalpy of 120 ± 1 J/g (n = 3; figure 2a)[19]. This result compares favourably with the previously reported range for the melting point (i.e. 160-191 °C) [20-21], although a higher

View Article Online
DOI: 10.1039/C8FO00729B

enthalpy (134 J/g)[20] has been reported in an earlier study. Preceding the melting peak of *'Fisher'* sucrose, a small endothermic peak with an enthalpy of 3.7 ± 0.4 J/g (n = 3) at 153.7 ± 0.09 °C (n = 3) was observed. The *'Silver Spoon'* sugar was also analysed using 6 replicate samples (figure 2b) and the curves indicated an onset melting peak at 189 ± 0.3 °C (n = 6) with an enthalpy of 131 ± 1.5 J/g (n = 6) but there was no indication of any endothermic peak at 153 °C. It has been postulated that the pre-melt endothermic peak was a direct result of the presence of an amorphous fraction or surface solubilisation of the crystals by residual moisture[23,24]. However, the occurrence of two endothermic melting peaks for the analytical grade sucrose has been reported by numerous independent investigators[25-30]. The observed difference in the number of peaks between the analytical grade and the commercial batch of sucrose may be attributed also to the presence of trace amounts of water or impurities e.g. salts, invert sugars (glucose and fructose) or organic acids which cannot be completely removed during the manufacturing process. The type and level of impurities, which vary among prepared sucrose samples by different manufacturing methods, may influence not only the number of endothermic peaks present, but also the melting temperature onset and the peak shape[28, 31-33]. The presence of such impurities can accelerate sucrose decomposition[26, 34-36]. It can be hypothesised that this extra pre-melt endothermic peak and the reduction of the melting peak enthalpy might be a consequence of the presence of invert sugars in the form of glucose/fructose or the existence of small amount of mineral salts introduced during sugar processing. In addition, as highlighted by Schmidt and co-workers[37], a pre-melting degradation may have a contributing factor, although the amounts of degradants observed in this later work are below 0.5% w/w and their presence occurred over long holding temperatures. Thus in the work reported here, holding and temperature cycling were avoided, but the authors still recognise the importance of impurities.

To investigate the impact of potential impurities on the DSC results, ion chromatography of the mixed standard solutions of sucrose, glucose and fructose was conducted. The chromatographic analysis demonstrated that *'Fisher'* and *'Silver Spoon'* sugar exhibited purity levels of 99.7 and 98.4% w/w respectively.

View Article Online
DOI: 10.1039/C8FO00729B

Low levels of impurities can be derived from monosaccharides (invert sugars), oligosaccharides (present in raw material - such as raffinose in beet or kestoses and theanderose in cane), polysaccharides (e.g. dextrans), and inorganic non-sugars (e.g. potassium chloride)[38]. The small endothermic peak apparent at ~ 150 °C (figure 2a) has been reported in a previous study to have persisted when sucrose was analysed in the absence of the invert sugars[24]. This suggests that it is not linked to the presence of fructose or glucose in the crystalline form of sucrose. Increasing the level of added invert sugar, has also been found not to significantly change the enthalpy of this peak[24,28]. The presence of the endothermic peak at ~ 150°C in the DSC curve of sucrose is likely to be linked to the mineral salt content of the sample, since adding mineral salts (Na and K) to the *'Fisher'* sucrose solution resulted in a complete removal of the peak at ~ 150 °C[28].

Minerals can be present in sucrose either due to the inorganic ash (mainly calcium and potassium salts) present in raw sugar or from occluded salts from the crystallisation media used in the production of sucrose. Therefore, the samples were run using a multi-element screen method which has the capacity to detect about 30 elements using an inductively coupled plasma mass spectrometry ICP-MS (Perkin Elmer DRC II, Software version 3.0) equipped with an autosampler. The potassium (K^+) and sodium (Na^+) ion content of *'Silver Spoon'* and *'Fisher'* sucrose was found to be 5.50 and 0.01 ppm (K^+) and 1.40 and 1.20 ppm (Na^+), respectively. Thus, the *'Silver Spoon'* sucrose contained a higher content of both K^+ and Na^+ ions compared to *'Fisher'* sucrose. Since the former grade of sugar is more representative of the commercial grade of sugar used in confectionary manufacture, it was decided to employ *'Silver Spoon'* sugar in preparing feed solutions intended for spray- or freeze drying.

Freeze-drying of 10% w/v aqueous sucrose feed solutions was performed according to the protocol reported in literature[8-12]. Freeze-drying was successfully applied to produce a relatively dry powder cake. The water content of freeze-dried sucrose was determined by TGA and was found to be 1.2 ± 0.3% w/w (n = 3), typical of the content found in milk chocolate crumb.

View Article Online
DOI: 10.1039/C8FO00729B

The OR of three replicates of sigmaUltra sucrose was measured and found to be 63.5 ± 0.6 ° (n = 3). *'Fisher'* sucrose was exhibited a specific OR of 63.5 ± 0.5 ° (n = 3) and 10% w/v *'Silver Spoon'* sucrose solutions shown to have a mean specific OR of 65 ± 1.1 ° (n = 3). Therefore, *'Fisher'* and sigmaUltra sucrose exhibited the same specific rotation values whereas, *'Silver Spoon'* sucrose was found to have a slightly higher value. The optical rotation (OR) of all prepared solutions was measured (Table 3).

The calibration curve of the specific rotation $[\alpha_{Obs}]_D$ vs sucrose concentration was linear over the range tested ($r^2 = 0.9981$). The freeze-dried sucrose sample produced an observed OR of 6.4°, corresponding to a specific rotation $[\alpha]_D = 64°$. This correlates to a purity level of 98.5% w/w, which suggested that no inversion occurred upon freeze-drying. This 1.5% reduction (from 100% to 98.5%) relates to the water content of the freeze-dried sucrose which was measured to be 1.2% w/w. Therefore, when this sample is dissolved in solution by weight, the actual concentration of sucrose in solution is effectively lower by this amount.

An overlay of three DSC replicates of amorphous freeze-dried sucrose illustrates that freeze-dried sucrose exhibits an initial step change corresponding to the T_g close to 50 ºC (Figure 3), which agrees favourably with the T_g range reported in literature[38]. The glass transition is followed by a peak corresponding to loss of water then a re-crystallisation exothermic peak is observed at ~ 120 ºC. Above the glass transition, the mobility of sucrose molecules increases dramatically and become sufficiently mobile to initiate nucleation and subsequent growth into macroscopic crystals[39]. As the temperature rises further, the sucrose sample starts to melt showing a melting peak at ~ 186-188 ºC followed by degradation (between 200 and 250 ºC)[24]. The DSC thermogram of freeze-dried sucrose confirmed the success of freeze-drying in producing a wholly amorphous sucrose sample. Low relative standard deviations (RSDs) of 1.7, 2.2 and 0.4% for the glass transition, re-crystallisation and melting point temperatures respectively, indicated a good repeatability of the DSC technique in confirming the amorphicity of sucrose. Despite the shape of the re-crystallisation peak being slightly variable, the area of this peak proved also to have acceptable repeatability, with an enthalpy of 79.0 ± 0.95 J/g being

View Article Online
DOI: 10.1039/C8FO00729B

obtained (Figure 3). The latter value correlated well with that reported previously[40]. Sucrose remained amorphous over a period of 6 months storage in dry conditions at 25 °C with the T_g values over that period not changing. The enthalpy of re-crystallisation also did not change during this time (~ 78 ± 1.6 J/g). The water content of the amorphous freeze-dried sucrose and freeze-dried sucrose containing 3% w/w NaCl by Karl-Fisher was 1.6 ± 0.07% w/w and 2.3 ± 0.1% (n = 3) respectively. Therefore by considering all of the experimental data discussed above, it could be concluded that stable amorphous sucrose was successfully produced with a high degree of purity by freeze-drying.

Hot stage microscopy images indicated a highly amorphous form of sucrose in the freeze-dried samples due to the lack of birefringence, typical of non-crystalline systems (Figure 4). As the samples were heated, a rearrangement of the molecules and re-crystallisation occurred, as shown by the appearance of birefringence (at 121 °C) due to the presence of crystals. Upon more heating, the whole sample melted and transformed to the liquid state. Therefore, hot stage microscopy indicated the presence of amorphous material in the freeze-dried sucrose mixtures. The DSC curve obtained for the heating of freeze-dried sucrose also confirmed the presence of a wholly amorphous sucrose sample (Figure 5).

It was also important to confirm the purity of lactose in the mixed sugar freeze-dried systems. The presence of sucrose has been reported to diminish the rate of lactose mutarotation, whereas sodium and potassium salts increase the rate[41,42]. However the accumulated evidence reported in literature has focused on the rate of mutarotation rather than final equilibrium ratio of the α- and β- anomers. The NMR results carried out using previously described methodology[15] , showed that for all freeze-dried sucrose/lactose mixtures (with a standing time of 4 h of the feed solution) the β/α enantiomer content of the lactose was always 60/40. This indicated that sucrose did not affect the final equilibrium ratio of the α : β-anomers of lactose.

When freeze-dried sucrose was subjected to DSC analysis using either pin-holed or hermetically sealed pans the samples exhibited very similar T_g values (~ 58 - 59 °C) (Figure 5). In contrast to the T_g °C, which remained constant, lower crystallisation onsets were detected for the

View Article Online
DOI: 10.1039/C8FO00729B

samples possessing residual water, (i.e. those present in hermetically sealed pans), in comparison to those where the residual water could evaporate through the pin-holed pans (Figure 5). For samples contained in the pin-holed pans a % RSD values (n = 6) of 1.9, 2.5 and 8.3% respectively were obtained for the glass transition, onset temperature of re-crystallisation and enthalpies of crystallisation. Whereas for samples in the hermetically sealed samples, the corresponding values for the % RSD (n=6) were 2.4, 2.5 and 11.6%.

The physical stability of amorphous sucrose was investigated by DSC over a period of 24 weeks (or six-months) to assess the efficiency of the storage conditions (Figure 6). Once freeze-drying was complete the sucrose samples were individually sealed under nitrogen and the sealed glass vials stored above phosphorus pentoxide at 25°C. This method proved successful as the DSC thermograms clearly indicate that amorphicity was maintained in all samples throughout the testing period, as demonstrated by the repeatability of the observed glass transition temperatures for all time points shown by a % RSD (n = 5) of 0.6% (Figure 6).

Enthalpic relaxation for the pure sucrose glasses was not observed, as seen by the absence of a small endothermic peak around the glass transition (Figure 6). This supports our approach of measuring the glass transition temperature for sucrose in the first heating scan and using the extrapolated midpoint method. It would have been difficult to apply a two heating scan approach, to erase any relaxation peaks present, as post melt degradation of sucrose is often recorded. These degradation products would be expected to affect the value of Tg.

When the freeze-dried amorphous sucrose containing 3% w/w NaCl was viewed on the hot stage microscope, it showed that the sample passed through the glass transition at 98 °C followed by re-crystallisation at 176 °C as determined by this method. Further heating caused the melting of the sample at 200 °C (Figure 7).

The glass transitions observed in the DSC occurred at lower temperatures when compared to those observed by hot stage microscopy. For example, the glass transition of amorphous sucrose containing 3% w/w NaCl fell between 52 and 55° C. In the case of amorphous sucrose alone the difference between the Tg values observed by hot stage

View Article Online
DOI: 10.1039/C8FO00729B

microscopy and DSC was much less, within 5° C. This small difference can be accounted for in terms of thermal contact between the sample and the heating elements present in a hot stage microscope, which is more pronounced when a highly porous material is under investigation. In addition, some difference is expected as the two approaches measure quite different physical properties; the DSC Tg is related to changes in capacity and the hot stage microscopy Tg measures the point where the super-cooled liquid begins to flow. However, this does not account for the pronounced difference observed in the Tg's measured by DSC and hot stage microscopy for the 3% w/w NaCl samples. The Tg's for the 3% w/w NaCl measured by DSC were 4 to 5°C lower than the Tg's for pure sucrose. This is as expected as small amounts of NaCl will lower the glass transition of many sugar glasses. Thus, the Tg's measured by DSC were considered here to be the true values. In the case of the NaCl containing sugar glasses and accounting for observations from the hot stage microscope, the super-cooled liquid above the glass transition must have been stiffer and avoided liquid flow until a much higher temperature than expected when compared to amorphous sucrose. The authors plan to test this hypothesis by measuring the mechanical properties of salt containing sugar glasses post Tg in future.

Comparing the DSC curves obtained for amorphous sucrose samples containing 3% w/w NaCl, contained in pin-holed with those placed in hermetically sealed pans then the same T_g (~ 52 - 55 °C) was obtained (Figure 8). However, a shift in the onset temperature of crystallisation was observed showing an earlier re-crystallisation onset temperature when heating was effected using hermetically sealed pans. When hermetically sealed sample pans were employed, the three samples exhibited a % RSD (n=3) of 2.4, 4.7 and 5.3% for the glass transition, onset temperature of re-crystallisation and enthalpy of crystallisation respectively. Whereas, for samples heated in the pin-holed pans, the corresponding values were 3.3, 5.3 and 10.7%.

An overlay of the DSC thermographs of freeze-dried sucrose only (with no NaCl or lactose) with those derived from freeze-dried sucrose containing 3% w/w NaCl in sealed pans is shown in Figure 9. The inclusion of NaCl lowered the T_g from 59.2 ± 1.4 °C to 52.3 °C ± 1.3.

View Article Online
DOI: 10.1039/C8FO00729B

Furthermore, both samples produced very similar onset temperatures of crystallisation (85 - 86 °C) while the enthalpy of re-crystallisation was lowered by NaCl from 54.8 ± 6.4 to 45.2 ± 2.4 J/g.

In pin-holed pans, the presence of NaCl lowered the T_g from 58.3 ± 1.1 to 54.8 ± 1.8 °C (Figure 10). In contrast, the onset temperature of crystallisation was increased from 122 ± 3.1 to 129.5 ± 6.9 °C; while the associated enthalpy of re-crystallisation was decreased from 72.8 ± 6.0 to 35.7 ± 3.8 J/g by NaCl. Therefore, the energy associated with the crystallisation of amorphous sucrose was halved in the presence of NaCl, when the samples were run in pin-holed pans i.e. minimal moisture content conditions. Moreover, the onset of melt degradation occurred at a much lower temperature in the presence of NaCl compared to the sucrose-only samples.

Hot stage microscopy of the freeze-dried sucrose: lactose (66:34% w/w) showed that as the sample passed the glass transition, it was transformed directly to the liquid state without re-crystallisation (Figure 11).

The DSC thermographs corresponding to sucrose samples containing medium levels of lactose & NaCl, i.e. 17% w/w lactose and 1.5% w/w NaCl, in hermetically sealed pans, had a mean T_g value of 56.4 ± 1.4 °C (n=3), whereas the same samples contained in pin-holed pans produced a mean T_g of 55.1 ± 1.4 °C (n=3). However, no re-crystallisation peaks were detected in these sugar mixtures thus the presence of either sugar hinders the re-crystallisation of the other, even at medium levels. NaCl can inhibit the crystallisation process of sugars by influencing either the solubility or melting point,which in turn affects the crystallisation process[39]. NaCl has been found to be significantly effective in inhibiting the crystallisation of mannitol from frozen systems[43], with such an effect being apparent at concentrations ranging from as low as 0.5 to 5% w/v; the higher the NaCl content, the harder it was for mannitol to crystallise. The presence of minerals has been shown previously also to influence the crystallisation temperatures and enthalpies of sugars[44]. Such an inhibitory effect of crystallisation induced by NaCl has been attributed to the tendency of NaCl to maintain water in hydration shells surrounding its composite ions, thus limiting any free water from contributing to the re-crystallisation process.

View Article Online
DOI: 10.1039/C8FO00729B

The freeze-dried sucrose samples containing high levels of lactose & NaCl i.e. 34% w/w lactose and 3% w/w NaCl possessed a T_g of 57.3 ± 2.3 °C (n=3) when presented to the DSC in pin-holed sample pans compared to 56.3 ± 2.3 °C (n=3) when the latter were hermetically sealed (Figure 12). Once more, no re-crystallisation was observed, confirming that the presence of lactose inhibits the re-crystallisation of amorphous sucrose[45]. Hermetically sealed pans versus pin-holed pans were used to mimic high residual water content versus low residual water content respectively. In samples containing low concentrations of lactose and NaCl, T_g was found to be minimally affected. However, the onset of crystallisation occurred at a much lower temperature in sealed compared to pin-holed pans and the enthalpy of crystallisation was lower in the former (Table 4). The sealed pans retain water in the sample while the pin-holed pans allow water to evaporate and the increased mobility of the molecules in the presence of water results in less energy being needed for the sucrose molecules to diffuse and orientate to join the forming and growing crystals.

Freeze-dried sucrose containing 34% w/w lactose only exhibited mean T_g values of 62.5 ± 4.5 and 61.0 ± 2.6 °C in pin-holed and hermetically sealed pans respectively (Table 4). The water content of these sucrose-lactose glasses fell within the range of 1.2 to 1.4 % w/w (data not shown). Again, no re-crystallisation peak was apparent in either case indicating that the amorphous sucrose and lactose did not crystallise. The DSC curves of amorphous sucrose also indicated that 17% w/w lactose eliminated any re-crystallisation in the pan. Thus, it appears that either sugar can inhibit the crystallisation of the other. This finding concurs with a previous report indicating that crystallisation rates were lower in mixed sugar system of sucrose and lactose than in a single sugar system; the crystal growth and diffusion of lactose into solution being inhibited by the presence of sucrose[9]. The impact of adding trehalose on the crystallisation of amorphous sucrose systems has been studied also where the shelf-life of pure sucrose systems can be very short[46,47]. Amorphous sucrose systems held above the glass transition temperature will collapse and crystallise but the addition of a small percentage of another type of sugar to sucrose, can extend the shelf-life of amorphous systems by slowing crystallisation. Raffinose has

View Article Online
DOI: 10.1039/C8FO00729B

been shown to slow down the crystallisation rate of sucrose in low moisture amorphous state systems[46,47]. Moreover, trehalose was found to interrupt the crystallisation of sucrose due to its higher glass transition compared to sucrose and the mechanism of inhibition can be explained by the attachment of the glucose units of one sugar to the major planar growing surface of the crystal. This can also apply to lactose that possesses a T_g that is higher than that of sucrose.

The factorial approach design of experiments is widely used in research[48-50]. The model employed comprises a standard least squares fitting (with an assumption that the responses were normally distributed) of the independent chosen variables (NaCl and lactose concentration, presence/absence of residual water) against each of the dependant variables (T_g, onset temperature of crystallisation or enthalpy of crystallisation). Therefore, this model was used to provide the predicted data values and then the predicted data were compared against the measured values using a simple linear regression. Goodness of fit and mean squared errors were used to check whether the model was capturing the trend in the experimental data[51].

With respect to enthalpy, the medium/medium results were excluded from the model as no data were obtained for the re-crystallisation process. The model exhibited an r^2 value of 0.92 which indicated that the proposed model fitted the experimental data well. The model showed a p-value < 0.0001, indicating a significant difference in the enthalpy of crystallisation (ΔH_{crys}) with respect to the samples with high versus low mineral content.

Including results for both pin-holed and hermetically sealed pans, the low NaCl freeze-dried samples generated a mean ΔH_{crys} = 63.9 J/g while the high NaCl freeze-dried samples had a mean ΔH_{crys} = 40.46 J/g. Therefore, the higher the level of NaCl, the lower the measured enthalpy of crystallisation from the amorphous starting forms. This was caused by incomplete crystallisation as there was amorphous sugar in the sample even after reaching the crystallisation temperature range.

When investigating the impact of the type of DSC pan, the statistical test produced a p-value of 0.183 indicating that sealing had no significant impact on the enthalpy of crystallisation. The high NaCl content samples when contained in sealed rather than pin-holed pans reduced the

View Article Online
DOI: 10.1039/C8FO00729B

difference between the means of ΔH_{crys} and these were no longer significantly different. Therefore, the low NaCl content samples in unsealed pans exhibited greater differences from all other samples, with the least square mean being much higher than for other samples. The interaction between NaCl levels and the sealed versus unsealed pans was statistically significant ($p = 0.001$) for ΔH_{crys}, with the measured mean value of the latter for low NaCl levels in unsealed and sealed pans being 72.8 and 54.8 J/g respectively. When samples were sealed, there was no significant difference in ΔH_{crys} between high and low NaCl content, nor between the values obtained for samples with a high NaCl presented in sealed and unsealed pans. The presence of NaCl also did not affect the onset temperature of crystallisation ($p=0.260$) but the sealing of the pans did influence the onset temperature of crystallisation ($p=0.001$). T_g was not affected by the NaCl concentration, the sucrose lactose ratio or whether the pan was sealed. ($p>0.05$).

4 Conclusion

It was concluded that a shortage of water during crystallisation in the headspace of the pan induces a higher crystallisation temperature T_{crys} in freeze-dried sucrose samples. NaCl also has an impact on the sugar (and/or recipes) by reducing the extent of crystallisation at a concentration of 3% w/w NaCl. The presence of lactose in the mix causes an interaction between lactose and sucrose, also inhibiting the crystallisation of both sugars. Therefore, the impact of different variables and interactions possible on the crystallisation of sucrose has been clearly addressed and understood. DoE has also proven to be a very efficient methodology, by saving time and resources, to investigate the correlations that may exist among different variables. Moreover, freeze-drying provides a good method for producing amorphous standards, as the chemical and physical purity may be maintained as compared to other techniques such as milling or spray-drying.

View Article Online
DOI: 10.1039/C8FO00729B

List of tables and figures

Table 1 A chocolate formulation showing typical percentages of components.

Component	Milk Chocolate % w/w
Cocoa mass (Cocoa solids and cocoa butter).	11.8
Added Cocoa butter	20.0
Sugar	48.7
Lecithin	0.4
Flavour Compounds	0.1
Whole Milk Powder	19.1
Total Fats	31.5

Table 2: Literature values of the glass transition of sucrose (Lappalainen *et al.*, 2005).

Glass transition of sucrose °C	Authors
48	Zeng *et al.*, 2001
52	Slade and Levin, 1991
57	Gloria and Sievert, 2001
66	Christensen *et al.*, 2002
67	Urbani *et al.*, 1997
67	Roos, 1993
70	Oxford *et al.*, 1990
72	Vanhal and Blond, 1999
74	Saleki-Gerhardt and Zografi, 1994
74	Shamblin *et al.*, 1996
77	Hancock *et al.*, 1995
78	Shamblin and Zografi, 1998
78	Shamblin *et al.*, 1999

Table 3 The optical rotation (and specific rotation ($[\alpha_{Obs}]_D$)) of solutions of *Silver Spoon* crystalline sucrose and its inverted forms.

Sucrose % w/w	Invert sugar % w/w (Glucose +Fructose)	Observed Optical Rotation °	$[\alpha_{Obs}]_D$	SD (n = 3)
100	0	6.5	65.4	0.3
75	25	4.9	49.8	0.3
50	50	3.2	31.9	0.1
25	75	1.1	11.4	0.4
0	100	-0.5	-5.2	1.1

View Article Online
DOI: 10.1039/C8FO00729B

Table 4 A summary of the different sugar samples with their corresponding T_g, onset temperature of crystallisation Tcrys, and enthalpies of crystallisation. The samples were labelled in a three letter format; where the first letter represents the level of NaCl, the second letter is the level of lactose and the third letter is the level of residual water; where L is low level, H is high level, M is medium level, S corresponds to hermetically sealed pans and U pin-holed pans.

Amorphous sucrose mixtures	**Tg °C (±SD, n=3)**	**Tcrys °C (±SD, n=3)**	**Enthalpy J/g (±SD, n=3)**
LLU	58.3 ± 1.1	122 ± 3.1	72.8 ± 6.0
LLS	59.2 ± 1.4	86.4 ± 2.2	54.8 ± 6.4
HLU	54.8 ± 1.8	129.5 ± 6.9	35.7 ± 3.8
HLS	52.3 ± 1.3	85.1 ± 4.0	45.2 ± 2.4
HHU	57.3 ± 2.3	-	-
HHS	56.3 ± 2.3	-	-
LHU	62.5 ± 4.5	-	-
LHS	61.0 ± 2.6	-	-
MMU	55.1 ± 4.1	-	-
MMS	56.4 ± 1.4	-	-

View Article Online
DOI: 10.1039/C8FO00729B

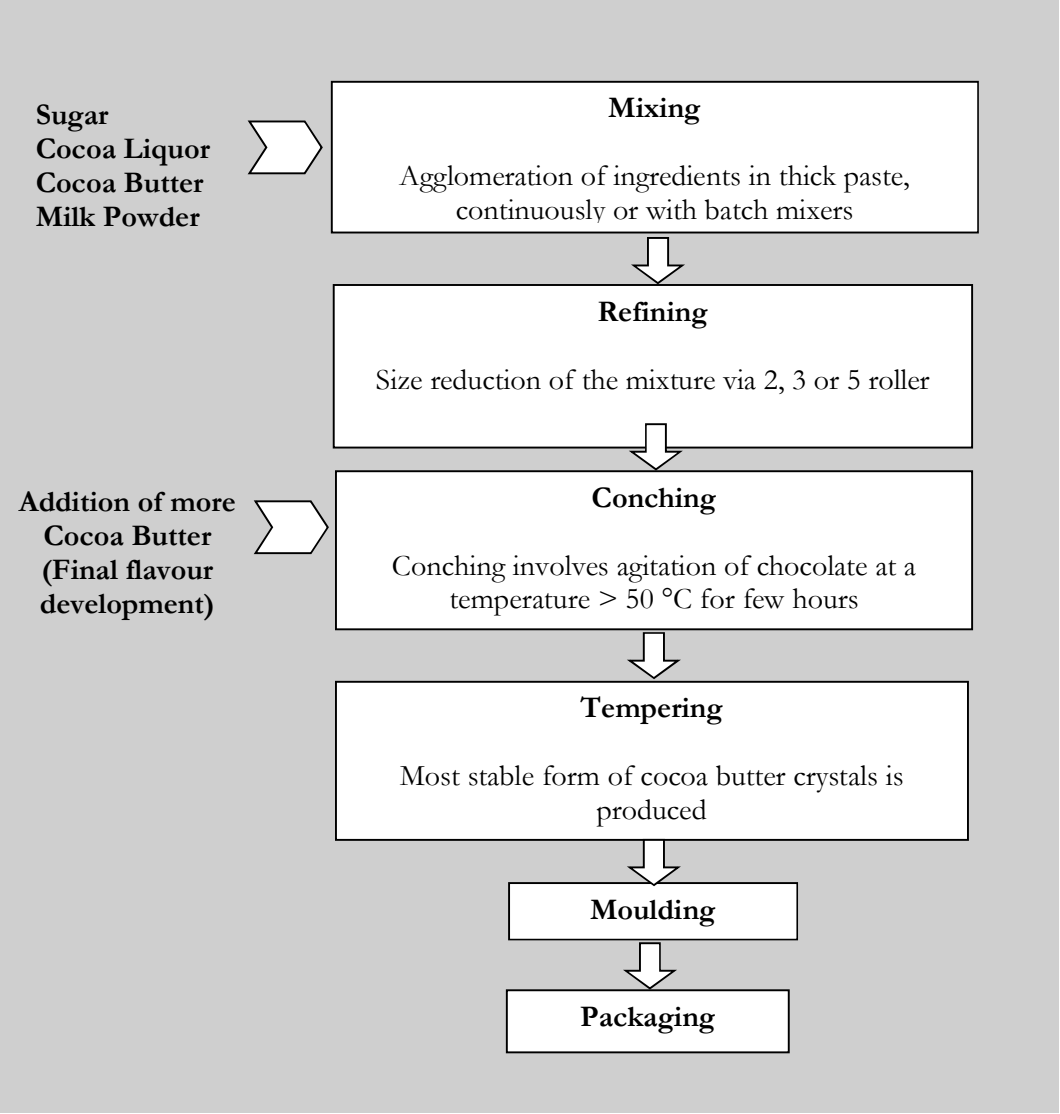


Figure 1 An overview of the chocolate manufacturing process of powder-based chocolate.

View Article Online
DOI: 10.1039/C8FO00729B

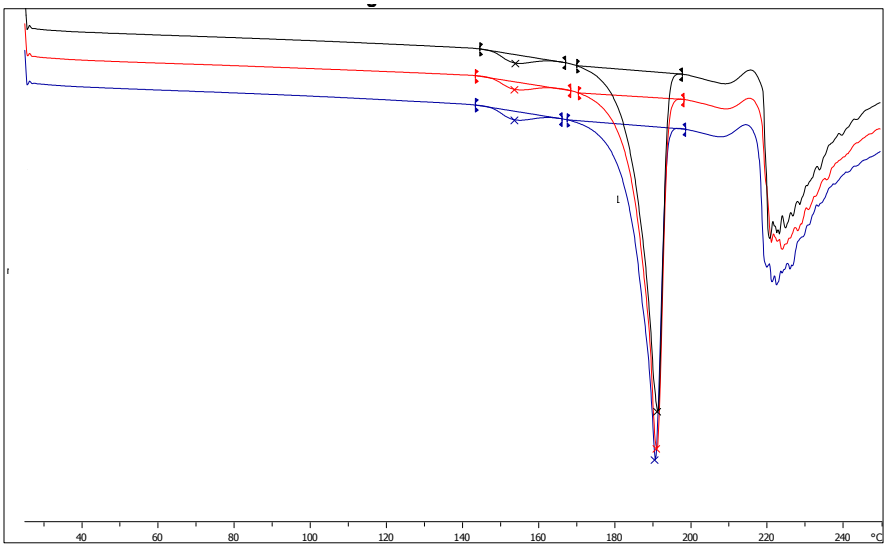


Figure 2a DSC curve of crystalline *'Fisher'* sucrose in pin-holed pans (triplicate) (Carrera, 2010), showing that the melting peak of Fisher sucrose is preceded by a small endothermic peak at 153.7 ± 0.09 °C (± SD, n = 3).

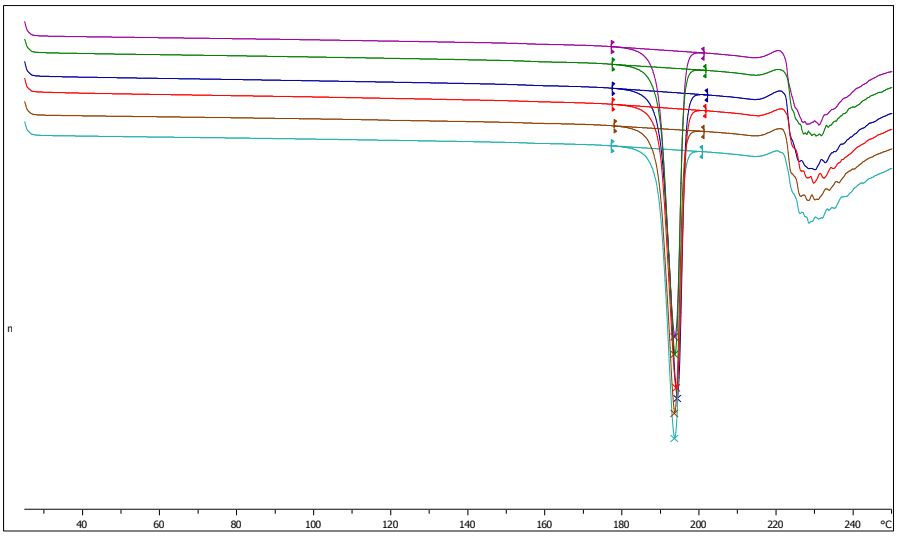


Figure 2b DSC curve of crystalline *'Silver Spoon'* sucrose in pin-holed pans (6 replicates) (Carrera, 2010) where there is no sign of any endothermic peak at 153 °C (preceding the melting point of sucrose).

View Article Online
DOI: 10.1039/C8FO00729B

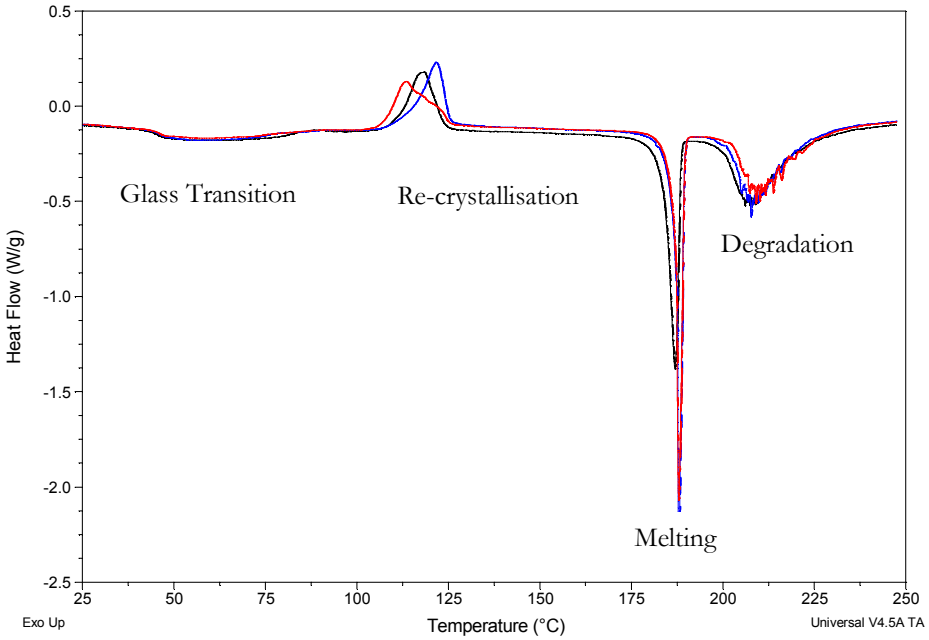


Figure 3 A DSC overlay of *'Silver Spoon'* freeze-dried sucrose in pin-holed pans (three replicates), showing the glass transition, re-crystallisation, melting peak and degradation at higher temperature.

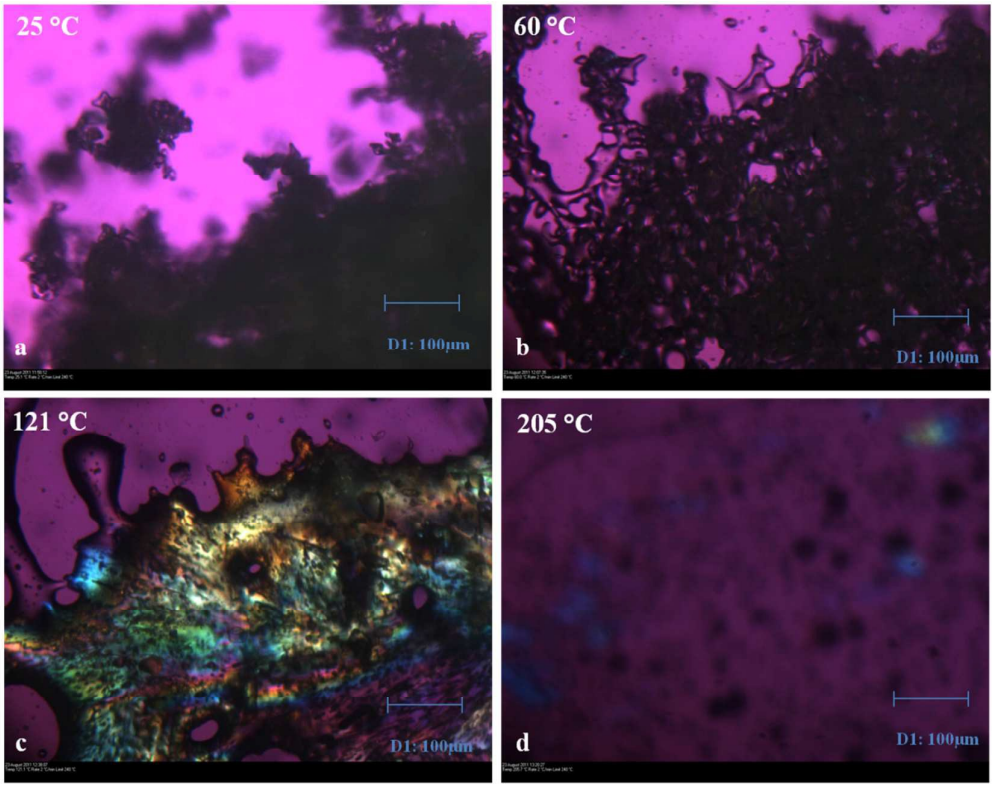


Figure 4 Hot Stage microscopy of freeze-dried sucrose (LL) a) 25 °C, below T_g b) 60 °C just above T_g c) 121 °C just at crystallisation d) 205 °C just above the melting point.

View Article Online
DOI: 10.1039/C8FO00729B

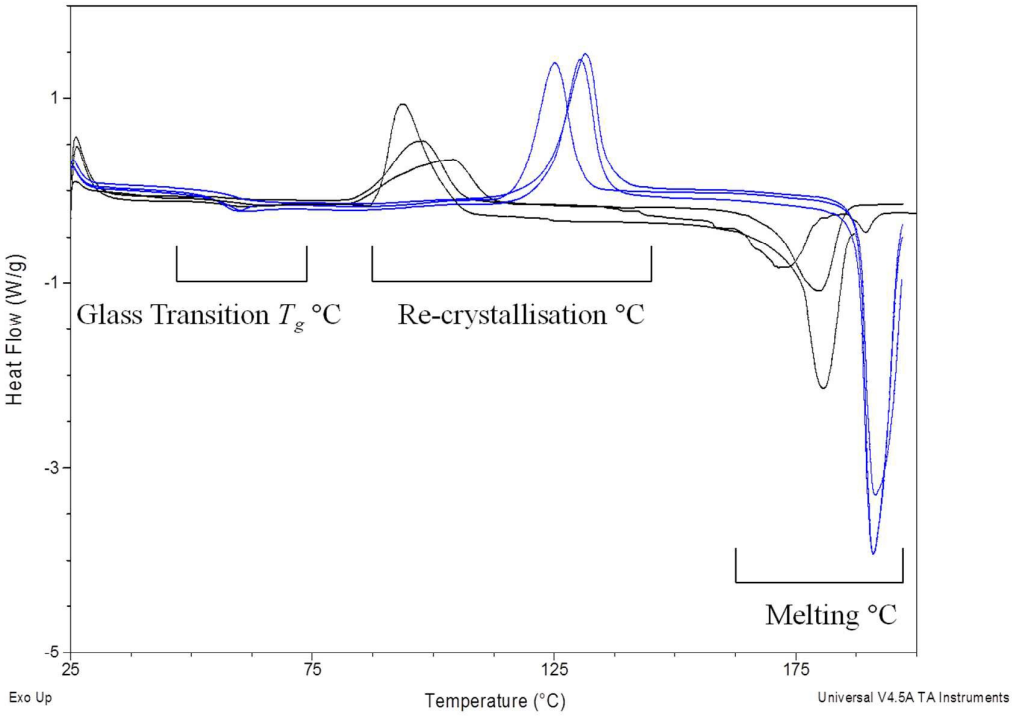


Figure 5 An overlay of freeze-dried sucrose samples heated in a hermetically sealed (black) compared with those heated in pin-holed pans (blue).

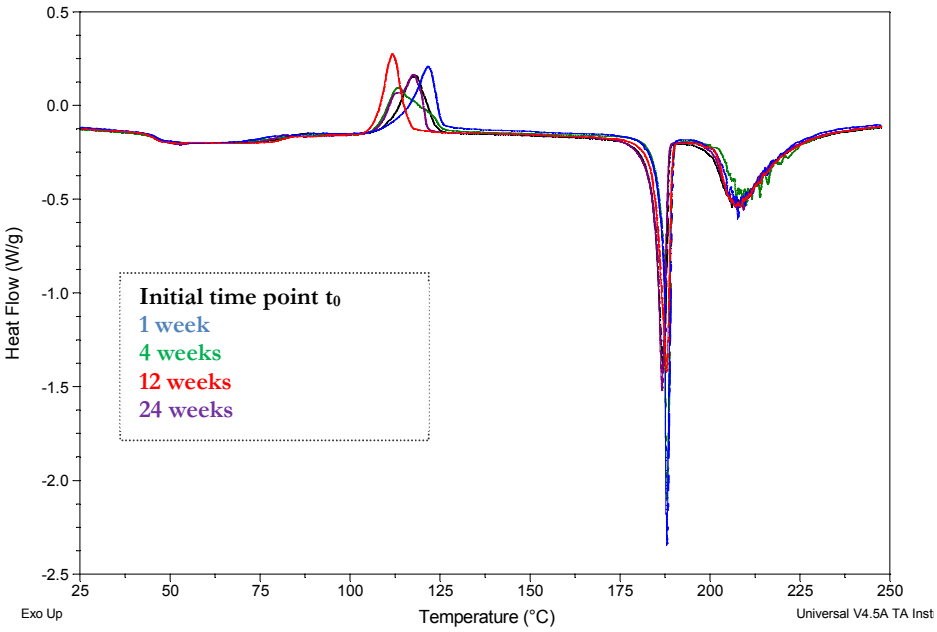


Figure 6 An overlay of DSC thermograms of freeze-dried sucrose samples at different storage time points (pin-holed pans).

View Article Online
DOI: 10.1039/C8FO00729B

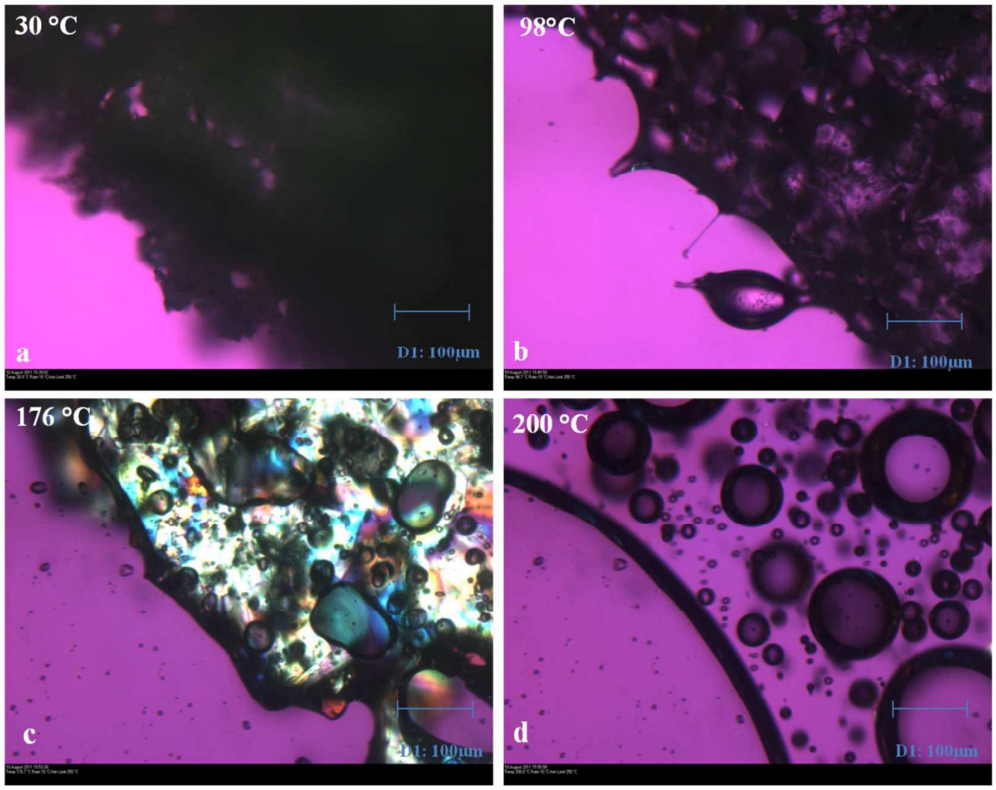
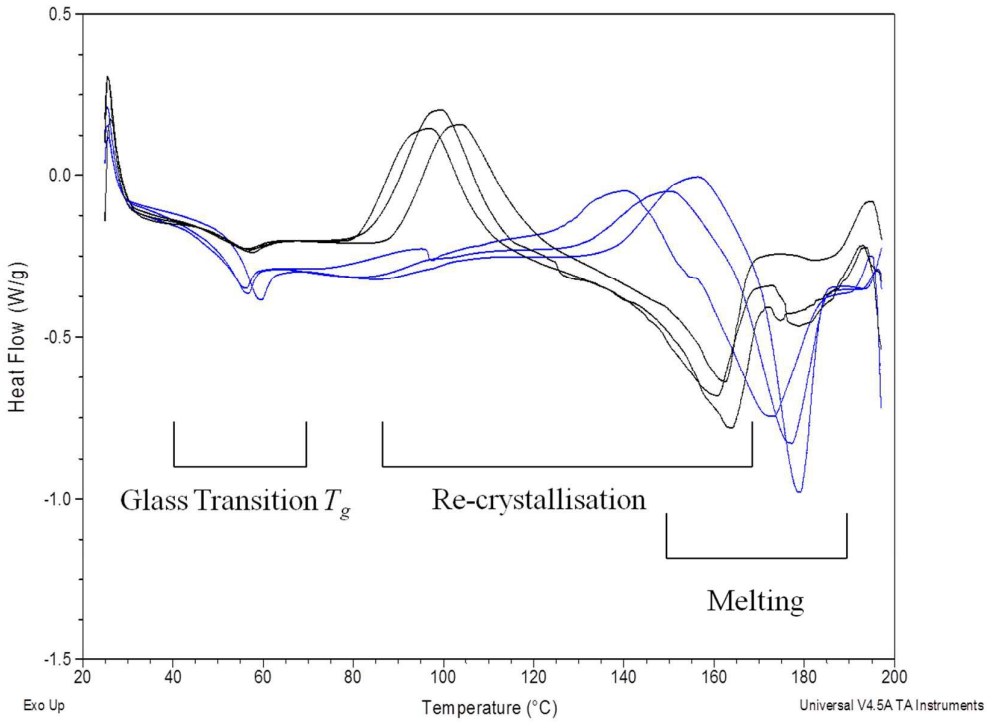


Figure 7 Hot Stage microscopy of freeze-dried sucrose containing 3% w/w NaCl a) 30 °C below T_g b) 98 °C above T_g c) 176 ° just above recrystallisation C d) 200 °C above melting point.



View Article Online
DOI: 10.1039/C8FO00729B

Figure 8 An overlay of freeze-dried sucrose samples containing 3% w/w NaCl heated in hermetically sealed (black) compared to similar samples heated in pin-holed pans (blue).

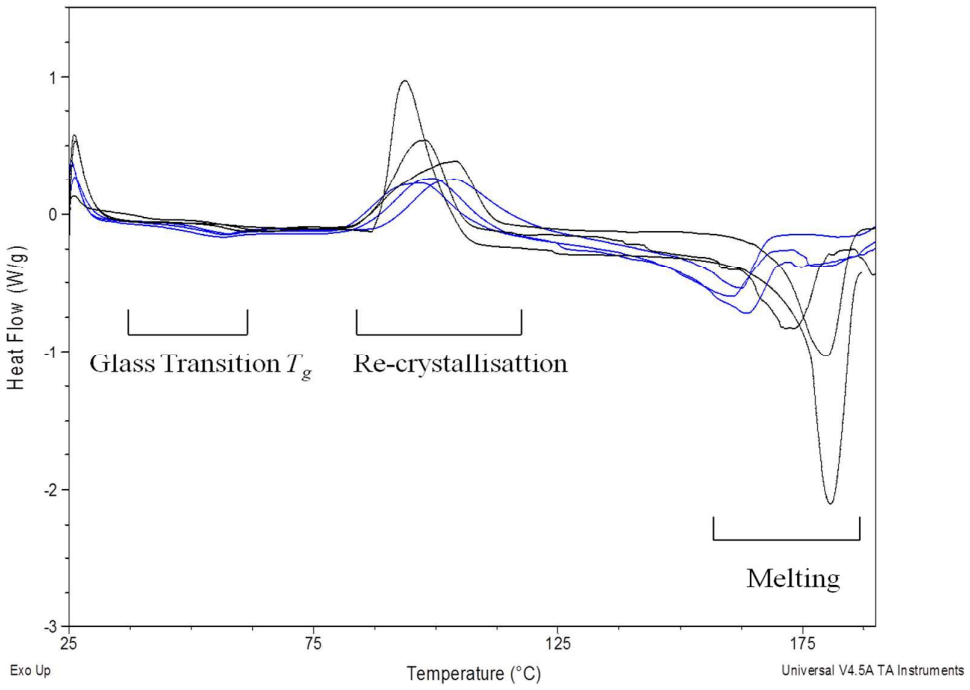


Figure 9 An overlay of freeze-dried sucrose samples (black) and freeze-dried sucrose with 3% w/w NaCl, both samples being heated in hermetically sealed pans (blue).

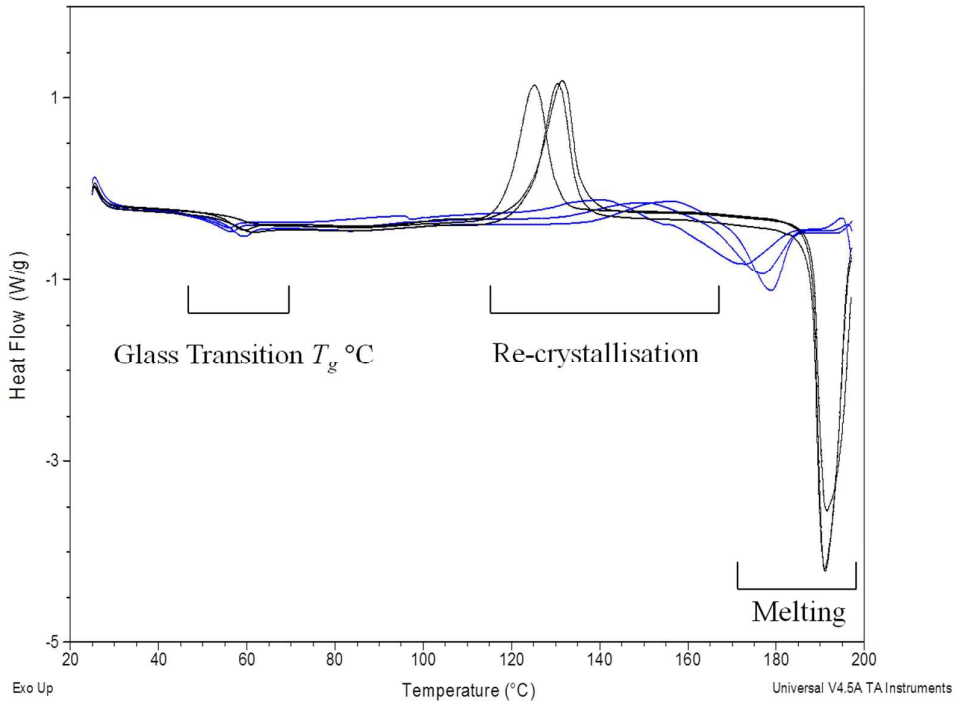


Figure 10 An overlay of freeze-dried sucrose samples (black) and freeze-dried sucrose with 3% w/w NaCl (blue), both samples being heated in pin-holed pans.

View Article Online
DOI: 10.1039/C8FO00729B

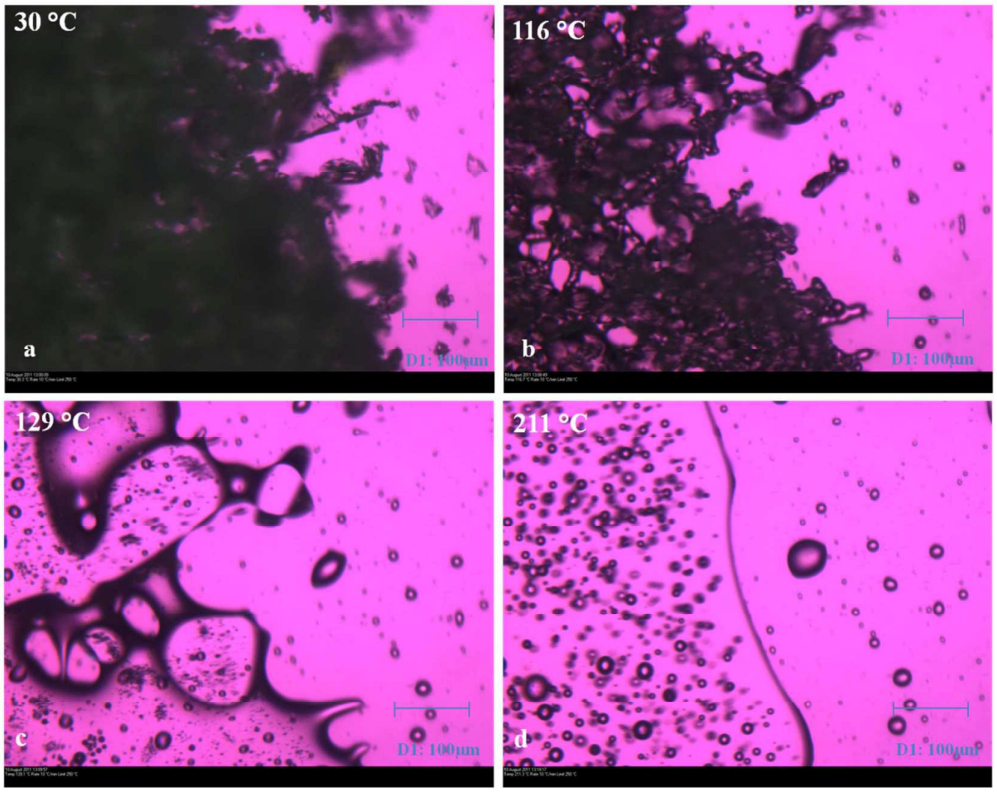


Figure 11 Hot Stage microscopy of freeze-dried sucrose containing 34% w/w lactose a) 30 °C below T_g b) 116 °C above T_g c) 129 °C liquid, no crystallisation observed d) 211 °C liquid.

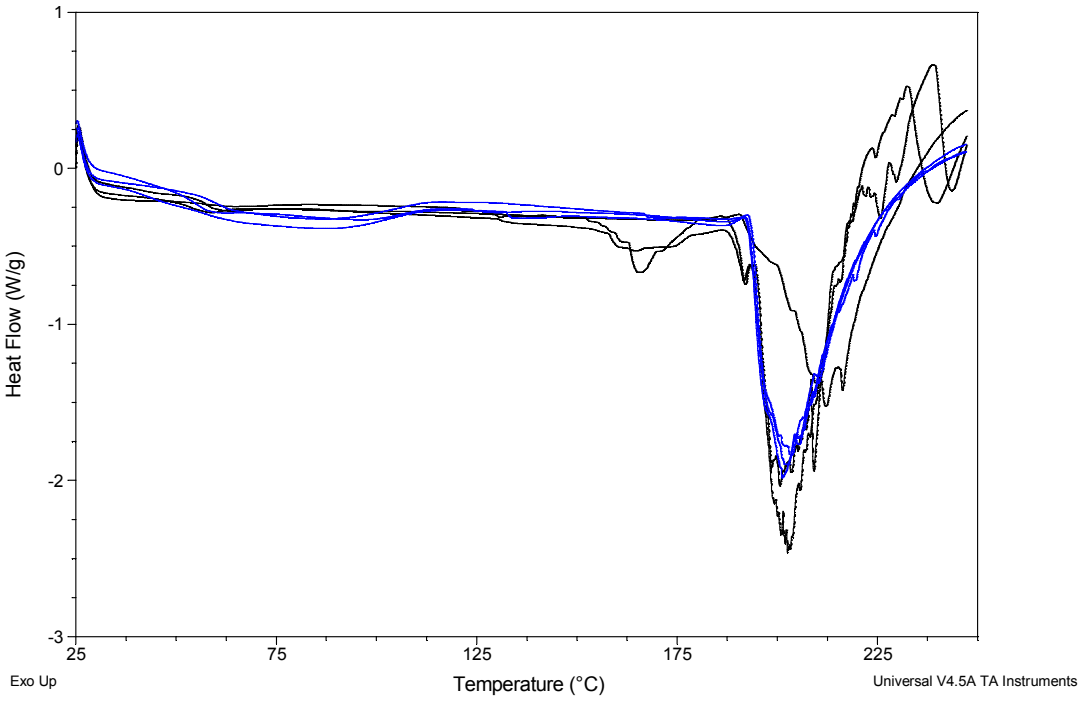


Figure 12 An overlay of freeze-dried sucrose containing 34% w/w lactose and 3% w/w NaCl in sealed (black) and pin-holed pans (blue).

View Article Online
DOI: 10.1039/C8FO00729B

References

1. Willart, J.F.; Caron, V.; Lefort, R.; Danede, F.; Prevost, D.; Descamps, M. "Athermal character of the solid state amorphization of lactose induced by ball milling" *Solid State Communications* 132 (2004) 693-696.
2. Chichester, C.O.; Schweigert, B.S. "Advances in food research" Academic Press (1988).
3. Markower, B; Dye, W.B. "Sugar crystallization, equilibrium moisture content and crystallization of amorphous sucrose and glucose" *Journal of Agricultural and Food Chemistry* 4: 1 (1956) 72-77.
4. Gaonkar, A.G.; McPherson, A. "Ingredients interactions" Second edition, CRC press (2006).
5. Attaie, H.; Breitschuh, B.; Braun, P.; Windhab, E.J. "The functionality of milk powder and its relationship to chocolate mass processing in particular the effect of milk powder manufacturing and composition on the physical properties of chocolate masses" *International Journal of Food Science and Technology* 38: 3 (2003) 325-335.
6. Hunter, N.E. "A spectroscopic and kinetic investigation into sugar glasses" thesis submitted to University of East Anglia (2009).
7. Islam, M.I.U.; Langrish, T.A.G. "An investigation into lactose crystallization under high temperature conditions during spray drying" *Food Research International* 43 (2010) 46-56.
8. Van Scoik, K.G.; Carstensen, J.T. "Nucleation phenomena in amorphous sucrose systems" *International Journal of Pharmaceutics* 58 (1990) 185-196.
9. Kedward, C.J.; McNaughton, W.; Blanchard, J.M.V and Mitchell, J.R. "Crystallization kinetics of lactose and sucrose based on isothermal differential scanning calorimetry" *Journal of Food Science* 63: 2 (1998) 192-197.
10. Chen, X. M.; Bates, S.; Morris, K.R. "Quantifying amorphous content of lactose using parallel beam X-ray diffraction and whole pattern fitting" *Journal of Pharmaceutical and Biomedical Analysis* 26: 1 (2001) 63-72.
11. Kawakami, K.; Miyoshi, K.; Tamura, N.; Yamaguchi, T.; Ida, Y. "Crystallization of sucrose glass under ambient conditions: evaluation of crystallization rate and unusual melting behavior of resultant crystals" *Journal of Pharmaceutical Sciences* 95: 6 (2006) 1354-1363.
12. Alkhamis, K.A. "Influence of solid-state acidity on the decomposition of sucrose n

View Article Online
DOI: 10.1039/C8FO00729B

amorphous systems II (effect of buffer)" *Drug Development and Industrial Pharmacy* 35 (2009) 408-416.

13. Neuss, J. D.; O'Brien M. G.; Frediani, H. A. "Sodium tartrate dihydrate as primary standard for karl fischer reagent" *Analytical Chemistry 23*: 9 (1951) 1332-1333.

14. Shugar, G.J.; Ballinger J.T. "Chemical technicians ready reference handbook" McGraw-Hill Inc., (1996).

15. Jawad, R.; Elleman, C.; Vermeer, L.; Drake, A.F.; Woodhead, B.; Martin G.P.; Royall, P.G. "The measurement of the β/α anomer composition within amorphous lactose prepared by spray and freeze drying using a simple ^{1}H-NMR method" *Pharmaceutical Research* 29:2 (2012) 511-524.

16. Morris, V.J.; Miles, M.J. "Physical techniques for the study of food biopolymers" ed S.B. Ross Murphy, Chapman of Hall New York (1994).
17. Cox, D.R.; Reid, N. "Theory of the design of experiments" (2000).

18. Tamhane, A.C. "Statistical analysis of *designed experiments: theory* and applications" (2009).

19. Carrera, J. "Determination of sugar crystallinity in chocolate formulations by DSC: an evaluation of thermal parameters" a thesis submitted to King's College London (2010).

20. Hurtta, M.; Pitkanen, I.; Knuutinen, J. "Melting behaviour of D-sucrose, D-glucose and D-fructose" *Carbohydrate Research* 339 (2004) 2267-2273.

21. Okuno, M.; Kishihara, S.; Otsuka, M.; Fujjii, S.; Kawasaki, K. "Variability of melting behavior of commercial granulated sugar measured by differential scanning calorimetry" *International Sugar Journal* 105 (2003) 29-35.

22. Roos, Y. "Melting and glass transitions of low molecular weight carbohydrates" *Carbohydrate Research* 238 (1993) 39-48.

23. Bhandari, B.R.; Datta, N.; Crooks, R.; Howes, T.; Rigby, S. "A semi-empirical approach to optimise the quantity of drying aids required to spray dry sugar-rich foods" *Drying Technology* 15: 10 (1997) 2509-2525.

24. Maulny, A.P.E.; Beckett, S.T.; Mackenzie, G. "Physical properties of co-crystalline sugar and honey" *Journal of Food Science* 70: 9 (2004) 567-572.

View Article Online
DOI: 10.1039/C8FO00729B

25. Richards, G.N.; Shafizadeh., F. "Mechanism of thermal degradation of sucrose: Apreliminary study" *Australian Journal of Chemistry* 31 (1978) 1825-1832.

26. Eggleston, G.; Trask-Morrell, B. J.; Vercellotti, J.R. "Use of differential scanning calorimetry and thermogravimetric analysis to characterize the thermal degradation of crystalline sucrose and dried sucrose-salt residues" *Journal of Agricultural and Food Chemistry* 44: 10 (1996) 3319-3325.

27. Eggleston, G.; Vercellotti, J.R.; Edye, L.A.; Clarke, M.A. "Effects of salts on the initial thermal degradation of concentrated aqueous solutions of sucrose" *Journal of Carbohydrate Chemistry* 15 (1996) 81-94.

28. Beckett, S.T.; Francesconi, G.M.; Geary, P.M.; Mackenzie, G.; Maulny, A.P.E. "DSC study of sucrose melting" *Carbohydrate Research* 341 (2006) 2591-2599.

29. Lee, T.; Lin, Y.S. "Dimorphs of sucrose" *International Sugar Journal* 109: 1303 (2007) 440-445.

30. Lee, J.W.; Thomas, L.C.; Schmidt, S.J. "Investigation of the heating rate dependency associated with the loss of crystalline structure in sucrose, glucose, and fructose using a thermal analysis approach (Part I)" *Journal of Agricultural and Food Chemistry* 59 (2011) 684-701.

31. Shah, S.V.; Chakradeo, Y.M. "A note on the melting point of cane sugar" *Current Science* 4 (1936) 652-653.

32. Hirschmuller, H. "Chemical properties of sucrose In Principles of Sugar Technology" First edition, Elsevier publishing (1953).

33. Kamoda, M. "Studies on the properties of sucrose crystal" *Research Society of Japan Sugar Refineries Technologists (SAUS)* 27 (1960) 258-238.

34. Kelly, F.H.C.; Brown, D.W. "Thermal decomposition and colour formation in aqueous sucrose solutions" *Sugar Technology Review* 6 (1978/79) 1-48.

35. Richards, G.N. "Initial steps in thermal degradation of sucrose" *International Sugar Journal* 88 (1986) 145-148.

36. Clarke, M. A.; Edye, L. A.; Eggleston, G. "Sucrose decomposition in aqueous solution, and losses in sugar manufacture and refining" A*dvances in Carbohydrate Chemistry and Biochemistry* 52 (1997) 441-470.

View Article Online
DOI: 10.1039/C8FO00729B

37. Lee, J.W.; Thomas, L.C.; Schmidt, S.J. "Investigation of the Heating Rate Dependency Associated with the Loss of Crystalline Structure in Sucrose, Glucose, and Fructose Using a Thermal Analysis Approach (Part I)" *Journal of Agricultural and Food Chemistry 59 (2011)* 684-701.

38. Roe, K. D.; Labuza, T.P. "Glass transition and crystallization of amorphous trehalose-sucrose mixtures" *International Journal of Food Properties* 8: 3 (2005) 559-574.

39. Hartel, R.W.; Shastry, A.V. "Sugar crystallization in food-products" *Critical Reviews in Food Science and Nutrition* 30: 1 (1991) 49-112.

40. Gloria, H.; Sievert, D. "Changes in the physical state of sucrose during dark chocolate processing" *Journal of Agricultural and Food Chemistry* 49: 5 (2001) 2433-2436.

41. Patel, K.N.; Nickerson, T.A. "Influence of sucrose on the mutarotation velocity of lactose" *Journal of Dairy Science* 53: 1 (1970) 1654-1658.

42. Bekkum, H. V.; Röper Harald, Voragen A. G. J. "Carbohydrates as organic raw materials III" Volume 3 (1996).

43. Telang, C.; Yu, L.; Suryanarayanan, R. "Effective inhibition of mannitol crystallization in frozen solutions by sodium chloride" *Pharmaceutical Research* 20: 4 (2003) 660-667.

44. Santagapita, P.R.; Buera M.P. "Electrolyte effects on amorphous and supercooled sugar systems" Journal of Non-Crystalline Solids 354 (2008) 1760-1767.

45. Livney, Y.D.; Donhowe, D.P.; Hartel, R.W. "influence of temperature on crystallization of lactose in ice-cream" *International Journal of Food Science and Technology* 30 (1995) 311-320.

46. Labuza, T.P.; Labuza, P.S. "Influence of temperature and relative humidity on the physical states of cotton candy" *Journal of Food Processing and Preservation* 28: 4 (2004) 274-287.

47. Leinen, K.M.; Labuza, T.P. "Crystallization inhibition of an amorphous sucrose system using raffinose" *Journal of Zhejiang University - Science B* 7: 2 (2006) 85-89.

48. Carmona, M.; Da Silva, M.; Leite, S. "Biosorption of chromium using factorial experimental design" *Process Biochemistry* 40 (2005) 779-788.

49. Bays, H.; Ose, L.; Fraser, N.; Tribble, D.; Quinto, K.; Reyes, R.; Johnson-Levonas, A.; Sapre, A.; Donahue, S.; and Ezetimibe Study Grp "A multicenter, randomized, double-

View Article Online
DOI: 10.1039/C8FO00729B

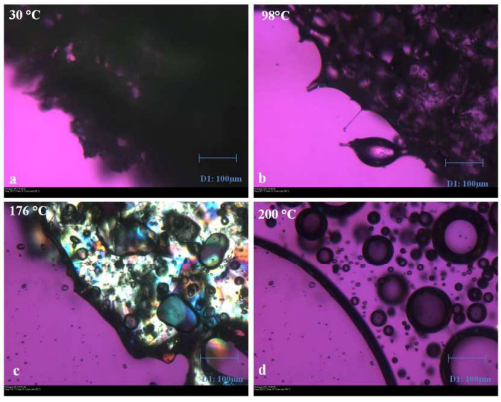
blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/ simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia" *Clinical Therapeutics* 26: 11 (2004) 1758-1773.

50. Gomez, K.A.; Gomez, A.A. "Statistical Procedures for Agricultural Research" (1984).

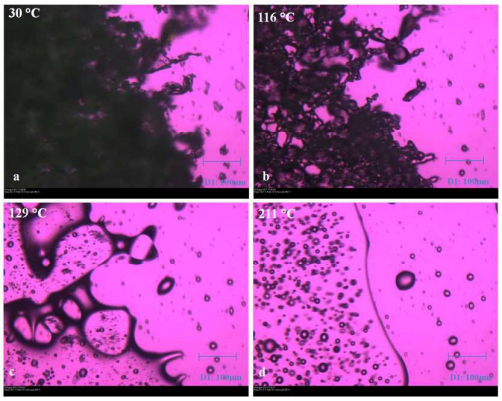
51. Wissmann, P.J. "Simultaneous approach to model building and process design using experimental design: application to chemical vapour deposition" submitted to Georgia Institute of Technology (2008).

View Article Online
DOI: 10.1039/C8FO00729B

Sucrose crystallization is highly dependent on the presence of other common food ingredients within an amorphous freeze-dried matrix.



NaCl reduces the glass transition temperature of amorphous sucrose



Lactose inhibits the crystallisation of amorphous sucrose